Esophageal and Gastric Cardia Cancer Risk and Folate- and Vitamin B_{12}-related Polymorphisms in Linxian, China

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Abstract

Linxian, a rural county in North Central China, has among the highest rates of esophageal squamous cell carcinoma (ESCC) and gastric cardia adenocarcinoma (GCA) in the world. Its inhabitants have documented chronic nutritional inadequacies, including folate and vitamin B_{12} deficiencies. Using a cohort we have been studying in Linxian since 1985, we examined the relationship between incident ESCC and GCA cancers and three polymorphisms in two genes that code for enzymes that require folate and B_{12} as cofactors: methionine synthase reductase (*MTRR*) *A66G* and methylenetetrahydrofolate reductase (*MTHFR*) *C677T* and *A1298C*. We conducted a case-cohort study among 4005 individuals in our cohort who were alive and cancer free in 1991 and had blood samples adequate for DNA extraction. Polymorphisms were measured on all 219 incident cancers (129 ESCCs and 90 GCAs) that developed through May 1996 and on 398 controls. Cox proportional hazard models were used to estimate relative risks (RRs) and 95% confidence intervals (CIs). Individuals with the *MTHFR 677TT* genotype had significantly higher combined ESCC/GCA risks (RR, 1.45; 95% CI, 1.02–2.05) than those with *CC* or *CT* genotypes. The only subjects to have *MTHFR 1298CC* were three ESCC cases ($P = 0.03$). Compared with subjects with the *MTRR 66AA* genotype, subjects with the *AG* or *GG* genotypes had significantly higher risk of ESCC (RR, 1.59; 95% CI, 1.04–2.42). No association was observed for GCA. Our results suggest that the *MTHFR C677T* and *MTRR A66G* polymorphisms influence the risk of ESCC and GCA in this population.

Introduction

Low folate has been associated with an increased risk for a number of gastrointestinal cancers (1), including esophageal (2–4) and stomach cancers (5–8). There are few studies examining vitamin B_{12} and esophageal or gastric cancer risk. One American study found a positive association between vitamin B_{12} intake and esophageal and gastric cancer (5). Several others found that subjects with pernicious anemia, a condition characterized by vitamin B_{12} deficiency, had greater risk for both cancers (9, 10). An ecological study in South Africa reported that subjects living in high ESCC[7] risk districts had significantly lower folate and B_{12} blood concentrations and nutrient intakes than did those living in low ESCC risk districts (11).

MTHFR and methionine synthase are two enzymes involved in folate and methyl group metabolism. MTHFR catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Five-methyltetrahydrofolate is the carbon donor for the major reaction that regenerates methionine from homocysteine. In addition to folate, this reaction requires methionine synthase and its coenzyme, B_{12}. MTRR maintains adequate concentrations of activated B_{12} for this reaction to occur. The integrity of these reactions has at least two important consequences. First, methionine in the form *S*-adenosylmethionine is the principle methyl donor for over 100 biological methylation reactions including DNA methylation. Second, the formation of methionine reconverts 5-methyltetrahydrofolate to tetrahydrofolate. Tetrahydrofolate is the precursor of 5,10-methylenetetrahydrofolate, which is required for the synthesis of nucleotides and purines for DNA. Deficiencies in folate and B_{12} and alterations in MTHFR, MS, and MTRR functions may contribute to carcinogenesis through altered DNA methylation (*e.g.*, DNA hypomethylation) and impeded thymidylate synthesis, resulting in nucleotide imbalances, increased uracil misincorporation in DNA, DNA strand breaks, and impaired excision repair, which may increase the susceptibility of DNA to mutations and damage (1).

Two common polymorphisms identified in the *MTHFR* gene have been associated with cancer (1). The best characterized is a missense mutation consisting of a $C \rightarrow T$ substitution at *bp 677* that produces an alanine to valine amino acid substitution within the predicted catalytic domain of the MTHFR enzyme. The second is a missense mutation consisting of an $A \rightarrow C$ substitution at *bp 1298* that produces a glutamate to alanine substitution. Several case-control studies from China

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[7] The abbreviations used are: ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; MTRR, methionine synthase reductase; MTHFR, methylenetetrahydrofolate reductase; RR, relative risk; CI, confidence interval; BMI, body mass index.

have observed that both *MTHFR 677TT* and *1298CC* variant genotypes were associated with ESCC (12), whereas only the *MTHFR 677T* allele was associated with gastric cancer (13–15). A more recently identified *MTRR* gene polymorphism involves an *A→G* substitution at *bp 66*, resulting in a methionine to isoleucine substitution. The variant *GG* genotype has been positively but nonsignificantly associated with colorectal cancer (16) and significantly associated with premature coronary heart disease, spina bifida, and Down's Syndrome (1).

Since 1985 we have been studying a cohort from Linxian, China, a rural county in North Central China, which has among the world's highest rates of ESCC and GCA. Originally, these individuals were participants in one of two randomized nutrition intervention trials conducted from 1986 to 1991, the General Population and Dysplasia Trials (17–19). The former included 29,584 subjects from four communes, and the latter included 3,318 subjects selected based on screening-detected cytologic esophageal abnormalities (17–20). Detailed descriptions of the trial designs, subject selection criteria, subject characteristics, methods of disease ascertainment, and primary endpoints have been published (17–20).

We recently measured pre-intervention levels of serum folate and B_{12} on a random sample of over 3000 individuals from this cohort, and we found that 90% of this population had marginal folate status (serum folate $<$6 ng/liter), and 75% had marginal B_{12} status (serum $B_{12} < 200$ pg/ml; Ref. 21). The goal of this current study was to examine the relationship between incident ESCC and GCA and three polymorphisms in two genes (*MTHFR C677T* and *A1298C* and *MTRR A66G*). Both genes produce enzymes for which folate and B_{12} are cofactors. Because the high rates for both cancers in Linxian suggest a common etiological factor, we examine risks separately for the end points ESCC and GCA as well as the combined end point ESCC/GCA (17–20).

Materials and Methods

Study Subjects. At the end of the Linxian intervention trials in 1991, approximately 6000 individuals who were alive and cancer free were selected for a blood sampling study. We were able to extract DNA yields of $\geq$1.5 μg from the RBCs on 4005 of these individuals. The subjects for this study were selected from this group ($n = 4005$), in accord with a stratified case-cohort design (22). The six stratums were defined by sex and the following three age categories: $<$50 years; 50–59 years; and $\geq$60 years. Polymorphisms we measured on all incident ESCC ($n = 131$) and GCA ($n = 90$) cases that occurred between May 1991 and May 1996 and on 421 controls. Excluded from this analysis are 22 individuals who could not be genotyped (2 ESCC cases and 20 controls) and 3 controls who lacked information on height and weight, leaving us with 129 ESCC cases, 90 GCA cases, and 398 controls. In each stratum, the control:site-specific case ratio was greater than 2:1. Disease classifications were based on monthly end point surveillance (17–20) and interview and examination of all living participants, or their next of kin, in May 1996 ($>$99% response rate). All cancer diagnoses from the 1991–1996 period were reviewed by our panel of United States and Chinese experts (20). Gastric cancers were defined as cardia cancers if they were in the proximal 3 cm of the stomach. These methods of assessment were identical to those used during the trial period (17–20).

Extraction of DNA and Genotyping. Genomic DNA was extracted from frozen blood, and the *MTHFR C677T* and *A1298C* and *MTRR A66G* polymorphisms were genotyped at a commercial laboratory (BioServe Biotechnologies, Ltd., Laurel, MD), using a PureGene kit (Gentra Systems, Inc., Minneapolis, MN) and real-time PCR analysis amplification (TaqMan; PE Biosystems, Foster City, CA). PCR primers and dual-labeled allele discrimination probes were designed using Primer Express software (version 1.5; Perkin-Elmer). All laboratory personnel were blinded to case-control status. A blinded repeat genotyping of 10% of the DNA samples yielded 100% concordance for all three polymorphisms.

Statistical Analysis. Information on age, sex, height, weight, BMI (kg/m^2), smoking (tobacco use for $\geq$6 months during lifetime: no, yes), alcohol use (any use during the past year: no/yes), and trial (Dysplasia or General Population Trial) were obtained from the 1985 questionnaire (17–20, 23). As reported previously (17–20, 23), this binary classification of smoking and drinking captures the variability of these characteristics in this population as assessed by more detailed measures. *P*s for case and control differences were generated using the Wilcoxon rank-sum (continuous variables) or χ^2 tests (categorical variables). In the one instance with small cell counts ($n \leq 5$), we used Fisher's exact test. Tests for Hardy-Weinberg equilibrium and tests and estimates of correlation between polymorphisms (Spearman correlation coefficients) were performed using only the 431 individuals in the "subcohort" (21, 24).

RRs and 95% CIs were estimated using a stratified case-cohort Cox proportional hazards models (21, 24). Models included indicator variables for trial, smoking, and alcohol use and continuous variables for BMI, and stratum-specific age. Risks are estimated separately for ESCC, GCA, and the combined category ESCC/GCA. Nested models were compared using score tests.

RR was first estimated with each genotype categorized by the number of variant alleles (0, 1, or 2). Trend tests were based on this classification. When indicated by similarity of risk estimate and/or biological considerations, we also estimated risks in groups defined by combining heterozygous individuals with one of the homozygous groups. For *MTHFR C677T*, we combined the *CC* and *CT* genotypes; for *MTHFR A1298C*, we combined the *AC* and *CC* genotypes; and for *MTRR A66G*, we combined the *AG* and *GG* genotypes. Effect modification of the polymorphisms with each other and for each polymorphism with the covariates in Table 1 was evaluated by the addition of cross product terms and within subgroup estimates.

Statistical Analysis Systems (SAS) software version 8.2 (Cary, NC) was used for the cross-sectional analyses, and Peanuts in Epicure (25) was used for the prospective analysis. All statistical tests were two tailed and considered significant at $P = 0.05$.

Results

Table 1 lists the baseline characteristic of subjects as measured in 1985 by case control status. Compared with the controls, ESCC and GCA cases were more often participants in the Dysplasia Trial. Female cancer cases had lower BMI than controls. When, as in the table, age was averaged overall strata, the ESSC cases were younger than the controls. However, when age comparisons are made within the six age-sex strata used in the analyses, no significant differences existed (data not shown).

Both *MTHFR C677T* ($P = 0.24$) and *MTRR A66G* ($P = 0.82$) were in Hardy-Weinberg equilibrium. *MTHFR A1298C* was not ($P < 0.005$): 0% had the *CC* genotype; whereas 1.7% was expected. The *MTHFR C677T* and *A1298C* polymorphisms were negatively correlated ($r = -0.37$, $P < 0.0001$). Fewer than 1% of subjects had both *MTHFR 677TT* and

Table 1 Baseline characteristics (1985) according to tumor location, Linxian Nutrition Intervention Trials and follow-up, 1991 through 1996

	Case subjects by tumor location			Control subjects (n = 398)
	Esophageal (n = 129)	Gastric cardia (n = 90)	Esophageal and gastric cardia (n = 219)	
Age (yrs)	51.0 (48.0–58.0)[a]	56.0 (50.0–61.0)	53.0 (48.0–59.0)	54.0 (50.0–59.0)
Sex (% male)	49.6	58.9	53.4	54.8
Height (cm)	159 (151–166)	160 (153–165)	159 (153–166)	158 (153–165)
Male	166 (162–169)	164 (160–167)	165 (161–168)	165 (160–169)
Female	151 (149–155)	153 (151–157)	152 (149–155)	152 (149–156)
Weight (kg)	52.0 (47.0–58.5)[a]	53.0 (48.0–59.0)	53.0 (47.0–59.0)[a]	54.0 (49.0–60.0)
Male	57.5 (52.0–61.0)	55.3 (52.0–60.0)	56.0 (52.0–61.0)	57.3 (53.0–61.5)
Female	48.0 (44.0–52.0)	49.0 (43.0–53.0)	48.0 (44.0–53.0)	50.0 (46.0–55.0)
BMI (kg/m^2)	20.8 (19.3–22.5)[a]	20.9 (19.4–22.2)[a]	20.8 (19.3–22.4)[a]	21.4 (20.1–22.9)
Male	20.8 (19.4–22.4)	20.9 (19.7–22.3)	20.8 (19.7–22.4)	21.3 (20.1–22.4)
Female	20.7 (19.3–22.5)[a]	20.6 (19.1–21.9)[a]	20.7 (19.3–22.5)[a]	21.5 (20.1–23.5)
Ever smoker (% yes)	37.2	47.8	41.6	38.7
Male	73.4	79.3	76.1	70.2
Female	1.5	2.7	2.0	0.6
Alcohol use during past 12 months, % yes	21.7	23.3	23.4	25.1
Male	40.6	34.0	37.6	39.0
Female	3.1	8.1	4.9	8.3
Dysplasia Trial participant (%)	58.1[a]	53.3[a]	56.2[a]	35.7

[a] Differed from non-case control subjects, χ^2 for categorical or Wilcoxon rank-sum for continuous $P \leq 0.05$.

1298AC/CC; no subjects had *MTHFR 677TT* and *1298CC* (data not shown). Neither of the two *MTHFR* polymorphisms were correlated with the *MTRR* polymorphism ($r = \sim 0$; *C677T*, $P = 0.39$; *A1298C*, $P = 0.14$).

Compared with *MTHFR 677CC* homozygotes, neither the *CT* nor *TT* genotype was associated with cancer (Table 2). With the combined *MTHFR 677CC/CT* genotypes used as referent category, the *TT* genotype was associated with a 1.45-fold increased risk for ESCC/GCA. Similar but nonsignificant risk increases were observed for separate ESCC and GCA. Only three individuals, all with ESSC, had *MTHFR 1298CC* (Fisher's exact $P = 0.03$). No associations were observed for the combined *MTHFR 1298AC/CC* genotypes. For *MTRR A66G*, ESCC risk increased with each additional variant G allele. The combined *AG/GG* genotypes were associated with a significant 1.59-fold increased ESCC risk. *MTRR A66G* was not associated

Table 2 Genotype frequencies, adjusted RRs, and 95% CIs for esophageal, gastric cardia, and combined esophageal and gastric cardia cancers and *MTHFR C677T, MTHFR A1298C, and MTRR A66G*, Linxian Nutrition Intervention Trials and follow-up 1991 through 1996

Genotype	Control subjects (n = 398)	Esophagus cases (n = 129)	RR[a] (95% CI)	Gastric cardia cases (n = 90)	RR[a] (95% CI)	Esophageal gastric cardia cases (n = 219)	RR[a] (95% CI)
	n (%)	n (%)		n (%)		n (%)	
MTHFR 677[b]							
CC	65 (16.3)	23 (17.8)	1.00 (reference)	17 (18.9)	1.00 (reference)	40 (18.3)	1.00 (reference)
CT	209 (52.5)	58 (45.0)	0.86 (0.48–1.54)	36 (40.0)	0.67 (0.35–1.29)	94 (42.9)	0.78 (0.49–1.26)
TT	124 (31.2)	48 (37.2)	1.24 (0.68–2.26)	37 (41.1)	1.17 (0.61–2.25)	85 (38.8)	1.21 (0.74–1.98)
			P-trend = 0.32[c]		*P*-trend = 0.30[c]		*P*-trend = 0.19[c]
MTHFR 677							
CC or *CT*	274 (68.8)	81 (62.8)	1.00 (reference)	53 (58.9)	1.00 (reference)	134 (61.2)	1.00 (reference)
TT	124 (31.2)	48 (37.2)	1.38 (0.89–2.11)	37 (41.1)	1.57 (0.98–2.50)	85 (38.8)	1.45 (1.02–2.05)
MTHFR 1298[d]							
AA	294 (73.9)	94 (72.9)	1.00 (reference)	69 (76.7)	1.00 (reference)	163 (74.3)	1.00 (reference)
AC	104 (26.1)	32 (24.8)	0.92 (0.57–1.48)	21 (23.3)	0.78 (0.45–1.34)	53 (24.2)	0.87 (0.59–1.28)
CC	0	3 (2.3)	Infinity	0	0	3 (1.4)	Infinity
			$P = 0.03$[e]				$P = 0.05$[e]
AC or *CC*	104 (26.1)	35 (27.1)	1.07 (0.76–1.51)	21 (21.8)	0.78 (0.45–1.34)	56 (25.6)	0.91 (0.62–1.34)
MTRR 66[f]							
AA	186 (46.7)	50 (38.8)	1.00 (reference)	43 (47.8)	1.00 (reference)	93 (42.5)	1.00 (reference)
AG	179 (45.0)	63 (48.8)	1.52 (0.97–2.37)	37 (41.1)	1.04 (0.64–1.69)	100 (45.7)	1.30 (0.91–1.85)
GG	33 (8.3)	16 (12.4)	1.94 (0.98–3.85)	10 (11.1)	1.22 (0.56–2.65)	26 (11.9)	1.59 (0.90–2.79)
			P-trend = 0.02[c]		*P*-trend = 0.66[c]		*P*-trend = 0.06[c]
AG or *GG*	212 (53.3)	79 (61.2)	1.59 (1.04–2.42)	96 (53.6)	1.07 (0.67–1.70)	126 (57.6)	1.35 (0.96–1.89)

[a] Adjusted for age, sex, trial, BMI, smoking, and alcohol use during past 12 months.
[b] χ^2 test *P* for *MTHFR C677T*: esophageal cancer, $P = 0.32$; gastric cardia cancer, $P = 0.09$; and combined esophageal/gastric cardia cancer, $P = 0.12$.
[c] Trends across three genotypes estimated using score tests from a continuous variable based on the number of variant alleles in each genotype.
[d] χ^2 test *P* for *MTHFR A1298C* and gastric cardia cancer, $P = 0.58$.
[e] Fisher's exact test *P*.
[f] χ^2 test *P* for *MTRR A66G*: esophageal cancer, $P = 0.18$; gastric cardia cancer, $P = 0.63$; combined esophageal/gastric cardia cancer, $P = 0.20$.

Table 3 Genotype frequencies, adjusted RRs and 95% CIs for esophageal, gastric cardia, and combined esophageal and gastric cardia cancers and *MTHFR C677T* and *MTRR A66G* by alcohol intake, Linxian Nutrition Intervention Trials

Cancer and genotype	Alcohol use during past 12 months[a]		*P*-interaction
	No	Yes	
MTHFR C677T			
Esophageal			
CC or *CT* (*n*)	65/210	16/75	
RR[b]	1.00 (reference)	1.00 (reference)	
TT (*n*)	36/100	12/31	
RR[b]	1.28 (0.78–2.11)	1.92 (0.80–4.58)	0.32
Gastric cardia			
CC or *CT* (*n*)	45/206	8/76	
RR[b]	1.00 (reference)	1.00 (reference)	
TT (*n*)	24/102	13/29	
RR[b]	1.19 (0.68–2.09)	5.32 (1.66–17.02)	0.03
Esophageal/gastric cardia			
CC or *CT* (*n*)	110/201	24/73	
RR[b]	1.00 (reference)	1.00 (reference)	
TT (*n*)	60/97	25/27	
RR[b]	1.25 (0.83–1.89)	2.82 (1.37–5.79)	0.04
MTRR A66G			
Esophageal			
AA (*n*)	35/154	15/45	
RR[b]	1.00 (reference)	1.00 (reference)	
AG (*n*)	53/131	10/51	
RR[b]	2.06 (1.23–3.45)	0.63 (0.25–1.58)	
GG (*n*)	13/25	3/10	
RR[b]	2.39 (1.08–5.30)	1.22 (0.26–5.66)	0.01
P-trend[c]	0.003	0.69	
Gastric cardia			
AA (*n*)	31/148	12/45	
RR[b]	1.00 (reference)	1.00 (reference)	
AG (*n*)	29/133	8/50	
RR[b]	1.21 (0.69–2.14)	0.81 (0.27–2.44)	
GG (*n*)	9/27	1/10	
RR[b]	1.49 (0.62–3.59)	0.90 (0.09–9.31)	0.49
P-trend[c]	0.32	0.75	
Esophageal/gastric cardia			
AA (*n*)	66/146	27/140	
RR[b]	1.00 (reference)	1.00 (reference)	
AG (*n*)	82/129	18/50	
RR[b]	1.66 (1.09–2.52)	0.69 (0.33–1.43)	
GG (*n*)	22/23	4/10	
RR[b]	1.94 (1.01–3.74)	1.20 (0.33–4.52)	0.08
P-trend[c]	0.008	0.63	

[a] Determined in 1985.
[b] Adjusted for age, sex, trial, BMI, and smoking.
[c] Trends across three genotypes estimated using score tests from a continuous variable based on the number of variant alleles in each genotype.

with GCA or combined ESCC/GCA. There was no evidence for interaction between the three polymorphisms and cancer risk.

Alcohol modified the associations of *MTHFR C677T* with GCA and combined ESCC/GCA and of *MTRR* with ESSC (*P* interaction = 0.03, 0.04, and 0.01, respectively). Among those with *MTHFR 677TT*, alcohol drinkers had approximately 5- and 2-fold the risk of GCA and ESCC/GCA as nondrinkers, respectively (Table 3). In contrast, alcohol drinkers with *MTRR 66GG* had approximately half the risk of ESSC as nondrinkers. There were no other significant interactions of the polymorphisms with the covariates in Table 1.

Discussion

We prospectively examined the relationship between three polymorphisms in two genes for enzymes for which folate and vitamin B_{12} are cofactors (*MTRR A66G* and *MTHFR C677T* and *A1298C*), and incident ESCC and GCA. In this Chinese population deficient in folate and vitamin B_{12}, we found that individuals with the *MTHFR 677TT* genotype had a 45% greater risk of ESCC/GCA than those with the *CC/CT* genotypes. This elevated risk was higher among alcohol drinkers. Although only three individuals had the *MTHFR 1298CC* genotype, all had ESCC (Fisher's exact $P = 0.03$). For *MTRR*, ESSC risk increased with each *66G* allele: those with one variant allele had twice the risk of the wild-type group, and those with two alleles had 2.4 times the risk of the wild-type group.

Several retrospective studies (12–15), all from China, have examined the association of the MTHFR polymorphisms and ESSC or gastric cancer. Concordant with our results, these studies found that individuals with the *MTHFR 677TT* genotype had increased risks of ESSC (Ref. 12; $n = 240$ cases) and GCA [Refs. 13 ($n = 82$ cases) and 14 ($n = 217$ cases)] of

approximately 6- and 2-fold, respectively. The only study to examine whether alcohol modified the *MTHFR 677TT* association was a case-control study of gastric cancer, which, although published only in Chinese, reports in its abstract a finding similar to ours. Given the sample size of our study and the number of interactions we examined, our findings on this alcohol effect are more suggestive than conclusive.

The association of *MTHFR 677TT* with malignancy is best studied in colon cancer. The research suggests that the *MTHFR 677TT* genotype is positively associated with cancer only among those with low methyl-group nutrient intake and folate status (1). The *MTHFR677T* allele produces a less stable thermolabile protein with lower MTHFR activity. This is thought to impair folate metabolism, particularly in those with deficiencies. The thermolability may contribute to carcinogenesis in a manner similar to folate deficiency by altering DNA methylation and/or thymidylate synthesis, thus resulting in nucleotide imbalances, uracil misincorporation into DNA, DNA strand breaks, and impaired DNA excision repair (1).

For the *MTHFR A1298C* polymorphism, the study of ESSC found a significant 4.43-fold increased risk with the *MTHFR 1298CC* genotype (12). The GCA studies found nonsignificant increased (14) or decreased risk (13). Although these results agree with ours, each study had a small number of subjects with the CC genotype, thus limiting the conclusions of any results.

To our knowledge, ours is among the first study to examine the relationship between the *MTRR A66G* polymorphism and cancer. Because *MTRR* requires B_{12} to support its function, and the majority of our population had marginal B_{12} status (21), it is possible that our population is particularly susceptible to perturbations in *MTRR*. Others have shown that the positive association between *MTRR 66G* and spina bifida offspring is stronger among mothers with low B_{12} status (1).

The greatest shortcoming of our study is the number of cancer cases observed. This limited our ability to examine the joint effects of the polymorphisms. The strength of our study is that it is prospective, based on a well-characterized cohort, with well-documented methods for end point assessment. Thus, it is free from the possible sampling, recall, and disease misclassification biases that can occur in case-control studies. Although studying a population with marginal folate and vitamin B_{12} status may have increased our ability to detect effects of the polymorphisms, it may also limit the generalizability of these results to Western populations.

In conclusion, we observed significant positive associations between the *MTHFR 677TT* genotype and combined ESCC/GCA and between *MTRR 66G* allele genotypes and ESCC in a folate- and vitamin B_{12}-deficient population. The association that we observed with the MTHFR polymorphisms is consistent with that of other studies in methyl-group-deficient populations. Our results suggest that the *MTHFR C677T* and *MTRR A66G* polymorphisms influence the risk of ESCC and GCA and support the hypothesis that folate and vitamin B_{12}, cofactors for their respective enzyme products, may play a role in the carcinogenesis of these cancers.

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